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Electrochemical techniques such as measurements of corrosion and redox potentials, polarization curves, polarization resistance, electrochemical impedance and electrochemical noise have been sued to evaluate the impact of marine microorganisms on corrosion processes. Surface analytical techniques including microbiological culturing, scanning, electron microscopy, microbes and microelectrodes have been used to characterize metal surfaces after exposure to marine waters. A combination of electrochemical, surface analytical and microbiological techniques is the most promising approach for determining mechanisms of MIC.

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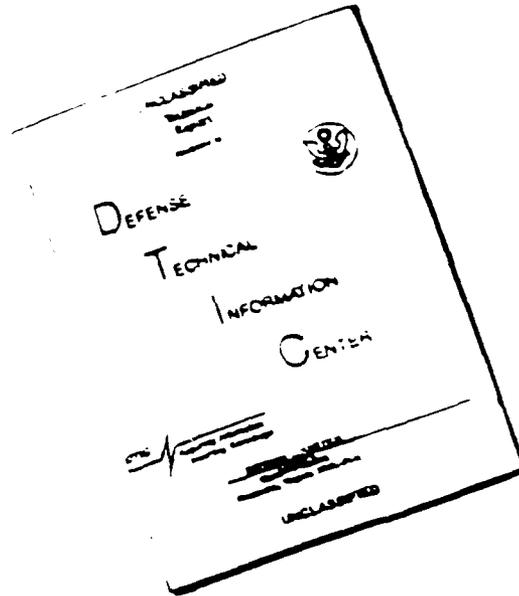
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in

Corrosion Research

Electrochemical Methods

in

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TEST METHODS FOR MICROBIOLOGICALLY INFLUENCED CORROSION (MIC) IN MARINE ENVIRONMENTS

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ABSTRACT

Electrochemical techniques such as measurements of corrosion and redox potentials, polarization curves, polarization resistance, electrochemical impedance and electrochemical noise have been used to evaluate the impact of marine microorganisms on corrosion processes. Surface analytical techniques including microbiological culturing, scanning electron microscopy, microprobes and microelectrodes have been used to characterize metal surfaces after exposure to marine waters. A combination of electrochemical, surface analytical and microbiological techniques is the most promising approach for determining mechanisms of MIC.

INTRODUCTION

In natural marine environments microorganisms attach to engineering materials, colonize surface and produce biofilms. The environment at the biofilm/substratum interface be radically different from that of the bulk marine medium in terms of pH, dissolved oxygen, organic and inorganic species. It is well documented that corrosion of metals alloys is more severe in natural seawater than in sterile chloride-containing media there is agreement among investigators that the increased corrosion is due to the presence of a biofilm [1-3]. Microbial activities within biofilms control the rates and of electrochemical reactions. The term microbiologically influenced corrosion (MIC) used to designate corrosion due to the presence and activities of microorganisms in biofilms at metal surfaces. MIC has received increased attention by corrosion tists and engineers in recent years due to the number of failures attributed to MIC the development of electrochemical and surface analytical techniques that can evaluate impact of microbes on corrosion phenomena. The following is an introduction to experimental techniques that have been used to evaluate MIC in marine environments

and should not be viewed as a complete review of the subject. Mansfeld and Little [4] recently published a review of electrochemical techniques applied to MIC.

TEST METHODS

Electrochemical Techniques

Corrosion and Redox Potentials

Time dependence of corrosion potential (E_{corr}) has been frequently monitored as a function of biofilm formation. Several investigators report an ennoblement or shift in the positive direction of E_{corr} for corrosion resistant metals during exposure in natural seawater and formation of a biofilm. Among the metals exhibiting this behavior are stainless steels (SS), copper alloys, titanium and platinum–Figures 1 and 2 [5–10]. Ennoblement of E_{corr} is usually explained by the acceleration of the cathodic oxygen reduction as a result of biofilm formation. Scotto et al. [7] and Mollica and Trevis [9] attributed E_{corr} ennoblement to organometallic catalysis of the oxygen reduction reaction and suggested that catalysts were formed by the reaction of extracellular polymers within the biofilm and metal species from the corroding metal. Johnsen and Bardal [8] observed ennoblement of non-corroding metals including titanium and platinum.

Mansfeld et al. [11] exposed several stainless steels and titanium to flowing Pacific Ocean water for time periods up to four months. A significant ennoblement of E_{corr} was not observed under any of the exposure parameters which included light and dark conditions and seasonal variations–Figure 3. Little et al. [12], exposing 304 stainless steel in Gulf of Mexico water, observed a slight positive shift of E_{corr} when biofilms
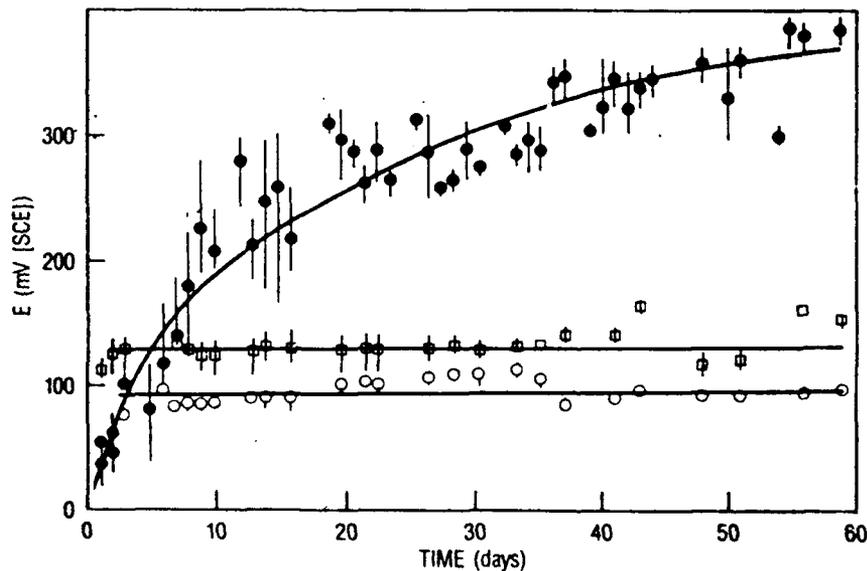
Figure 1. Open circuit corrosion potential curves as a function of time for 21 Cr – 3 Mo steel exposed in natural and artificial seawater. Natural seawater (●); autoclave-sterilized artificial seawater (□); and filtration-sterilized natural and artifical seawater (○) [7].
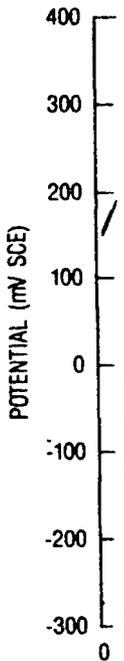
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Figure 2. Open circuit potential vs. time for stainless steel 254 SMO, platinum, titanium and 90-10 CuNi in seawater [6].

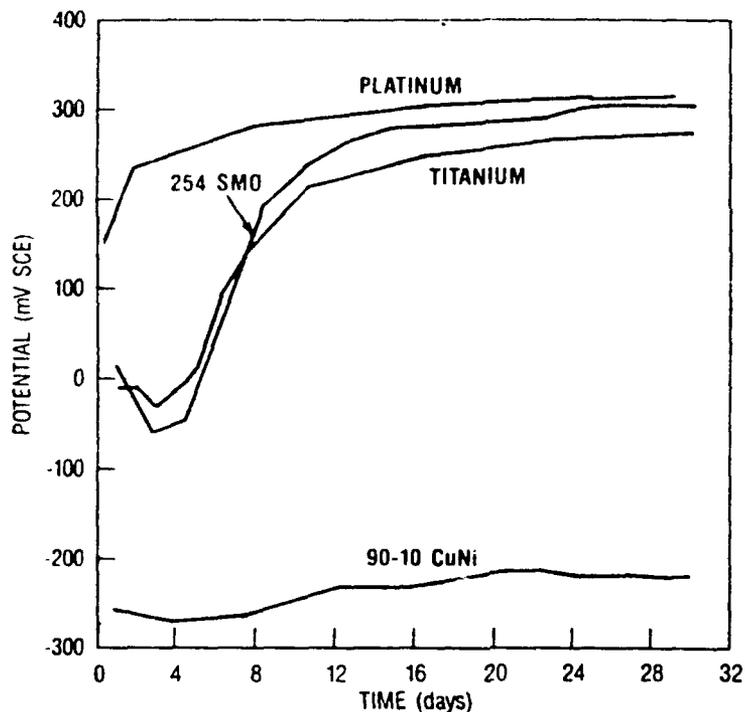developed under fully illuminated conditions. Using microelectrodes for pH and dissolved oxygen, they determined that during ennoblement the biofilm/metal interface was aerobic and that interfacial pH was unchanged from that of seawater. Biofilms that developed in the presence of light were dominated by diatoms. Diatoms are microscopic algae with siliceous cell walls that modify interfacial chemistry by influencing local oxygen concentration, changing the pH and producing aggressive metabolites [13]. Motoda et al. [14] demonstrated an ennoblement of stainless steel of 200–300 mV in the presence of an axenic culture of photosynthetic diatoms. Dowling et al. [15] observed a positive shift of E_{corr} (2–15 mV) for a stainless steel exposed to photosynthetic bacteria in the presence of light–Figure 4. This small increase of E_{corr} agrees with the dependence of the reversible potential of the oxygen electrode on the partial pressure of oxygen.

Videla et al. [10], Ringas and Robinson [16] and Gouda et al. [17] demonstrated a decrease of E_{corr} for a variety of stainless steels and copper alloys in seawater media and in pure and mixed cultures of anaerobic sulfate-reducing bacteria (SRB). Little et al. [12] measured highly localized areas of pH ranging from 5.2 to 9.2 at anaerobic biofilm/metal interfaces where E_{corr} was shifted in the negative direction. Gomez de Saravia et al. [18] reported E_{corr} measurements for copper/nickel alloys incubated in artificial water with cultures of facultative aerobic bacteria (*Vibrio aicmolyticus*) and an anaerobic sulfate-reducing bacterium (*Desulfovibrio vulgaris*)
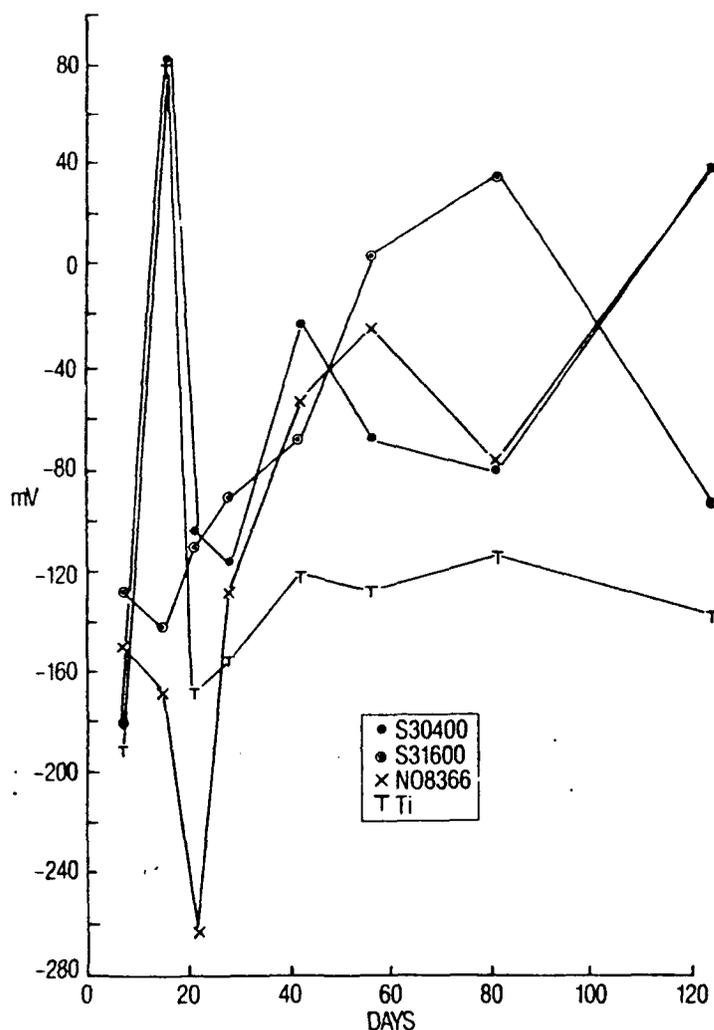
Figure 3. Open circuit potential vs. time for 3 stainless steels and
titanium exposed to natural flowing seawater [11].

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Figure 5a and 5b. A displacement of E_{corr} in the negative direction with respect to the
sterile control was measured during growth of *V. alginolyticus*. When *D. vulgaris* was
added E_{corr} was further depressed. Little and Mansfeld [19,20] did not observe signifi-
cant changes of E_{corr} for copper alloys during exposure to Pacific Ocean water.

Shifts of E_{corr} can be due to either thermodynamic or kinetic effects. In terms of
thermodynamic effects, localized acidification or an increase of the partial pressure of
oxygen pO_2 would shift the reversible potential of the oxygen electrode in the positive
direction. Increasing pO_2 leads to very small increases of E_{corr} as observed by Dowling
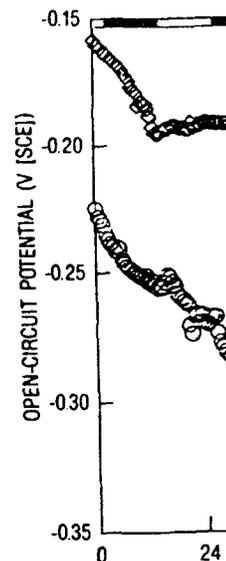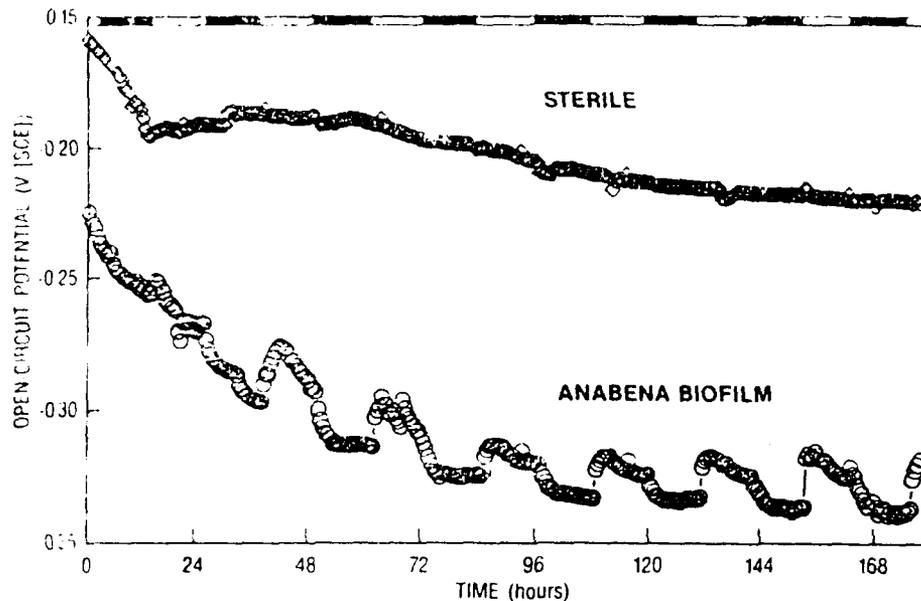et al. [15] with photosynthetic bacteria.

Figure 4. Open circuit potential vs. time for 316L stainless steel using photosynthetic bacteria in presence of light [15].

The role of local acidification due to microbiologically produced acids has also been considered as a possible mechanism for ennoblement of E_{corr} [5]. In addition to direct production of acidic metabolites, microorganisms can indirectly influence local pH by accelerating cathodic and anodic reactions. The cathodic reduction of dissolved oxygen results in an increase of pH at the metal/electrolyte interface [21]. If microorganisms accelerate dissolution of a metal at anodic sites, metal cations are formed which will be hydrolyzed by water with the formation of H+ ions:

$$M \Rightarrow M^{2+} + 2e \tag{1a}$$

$$M^{2+} + 2H_2O \Rightarrow M(OH)_2 + 2H^+ \tag{1b}$$

causing a decrease in interfacial pH. Kinetic effects due to catalytic reactions would lead to an increase of the exchange current density i_o for the oxygen reduction/oxidation reaction and an increase of the rate of the oxygen reduction at a constant potential in the charge transfer-control region.

Redox potential measurements are frequently used to assess the likelihood of growth of SRB. SRB require reducing conditions (typically below -400mV $_{SHE}$). Zhang et al. [22] compared E_{corr} values for a prepassivated platinum electrode and a 304 stainless steel electrode with and without bacteria to follow development of a biofilm–Figures 6a and 6b, respectively. The platinum electrode was used as a redox electrode to indicate changes in the local oxygen concentration which were assumed to be the same as those on the stainless steel. Since E_{corr} for platinum changes in the positive direction when
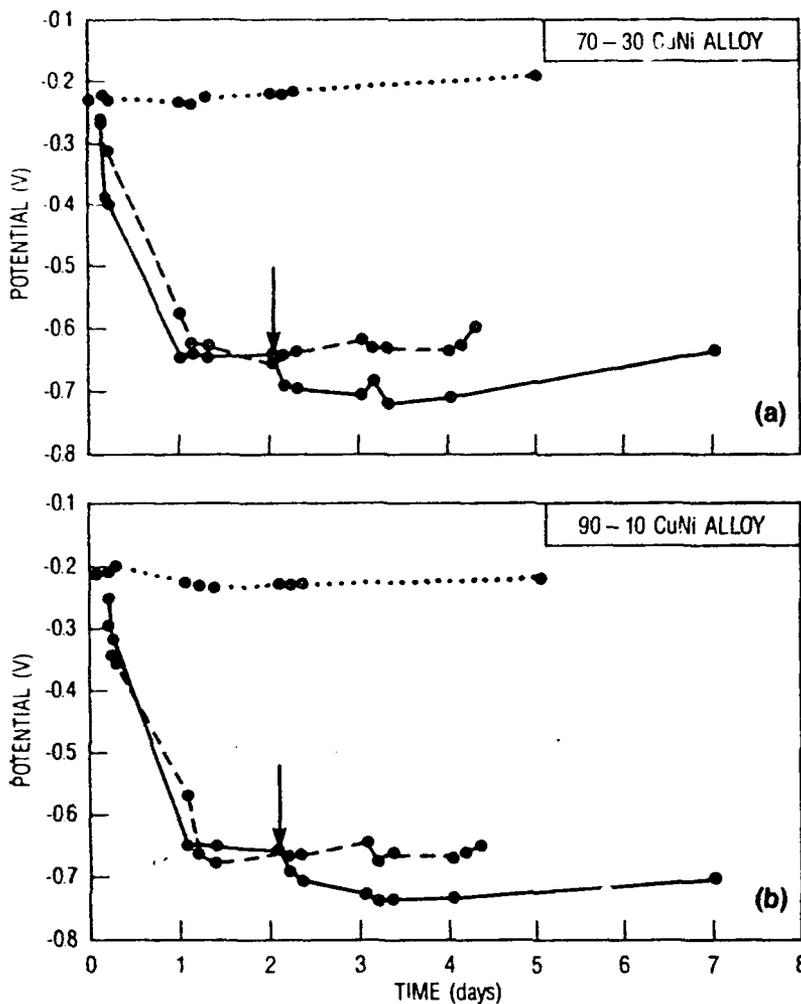
Figure 5. Corrosion potential vs. time ((a) 70-30 Cu/Ni alloy, (b) 90-10 CuNi alloy) in Postgate ∪ medium + 0.51 M NaCl inoculated with V. alginolyticus (----); Postgate C medium + 0.51 NaCl inoculated with V. alginolyticus and 48 hours later with D. vulgaris (arrow) (——) and sterile control (····)[18].

either the oxygen concentration is increased or pH is decreased, a pH indicator was added to the solution to independently determine pH changes. During the 72-hour exposure of the stainless steel to bacteria E_{corr} fluctuated with changes in microbial activities. Lewandowski et al. [21] developed microelectrodes for measuring the redox potential within the biofilm thickness. To date those measurements have not been coupled to specific electrochemical measurements.

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Figure 6. Open circuit potential vs. time for metal alloys in
sterile and bacterial culture media (a) platinum and (b) 304SS
[22]

Polarization Techniques

Polarization techniques assume a uniform corrosion process in which oxidation and reduction reactions occur randomly over a surface with regard to both space and time. Biofilms are patchy in their distribution and composition, giving rise to localized anodes and cathodes. The model of uniform corrosion is difficult to apply directly to MIC.

Numerous investigators have recorded polarization curves to determine the effects of microorganisms on electrochemical properties of metal surfaces in marine environments and the resulting corrosion kinetics. In most of these studies comparisons were made between polarization curves recorded in sterile media with those obtained in the presence of bacteria and fungi. Deshmukh et al. [24] evaluated the influence of sulfide on naval brass in polluted seawater using potentiodynamic polarization curves–Figure 7. The shape of the anodic polarization curve changed drastically and E_{corr} became more negative in the presence of SRB or dissolved su l ˌ active-passive transition and hysteresis

*Figure 7. Potentiodynamic polarization curves for naval brass
in seawater media [24].*

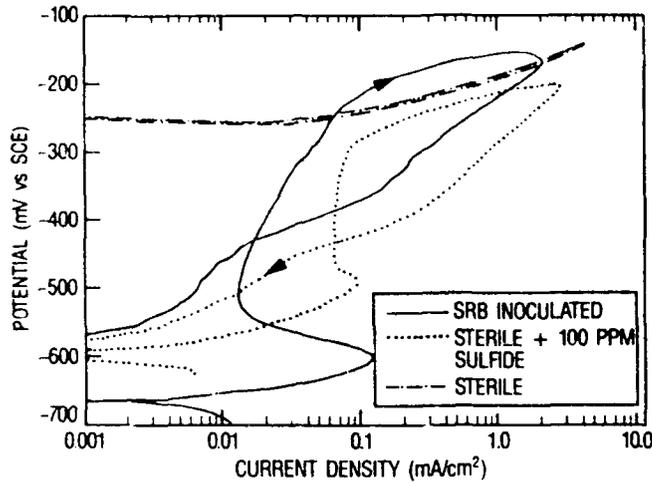in the reverse scan were observed. Castle et al. [25] used polarization techniques to demonstrate that copper-nickel alloys were susceptible to transient reductions in oxygen level in natural seawater, but not in sodium chloride solutions. These results were attributed to organic components within the biofilm. The key to good performance of copper/nickel alloys is the inhibition of the cathodic reduction of oxygen. The presence of organic material at the metal interface provides other species for reduction after the metal biofilm interface becomes anaerobic.

Pitting potential (E$_{pit}$) derived from polarization measurements has been used to assess the susceptibility of metals and alloys to localized corrosion in microbiologically modified environments. E$_{pit}$ is usually determined from an anodic polarization curve as the potential value at which the current density begins to increase dramatically. E$_{pit}$ is defined as the potential above which pits can initiate and grow. For stainless steels in NaCl, E$_{pit}$ is given by:

$$E_{pit} = a - b \log a_{Cl^-} . \tag{2}$$

where a and b are experimentally determined parameters [26]. For 304SS the dependence of E$_{pit}$ on the activity of the chloride ion a$_{Cl^-}$ has been demonstrated:

$$E_{pit} = -0.074 - 0.088 \log a_{Cl^-} \ (V \ vs. \ SCE) . \tag{3}$$

The probability of localized corrosion increases as E$_{corr}$ shifts in the positive direction into the range of E$_{pit}$. The difference between E$_{pit}$ and E$_{corr}$ ($\Delta E = E_{pit} - E_{corr}$) has been used as an indication for susceptibility to pitting.

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Table 1. E_{pit} variation of Al 2024 with chloride concentration, pH and microorganism growth. The composition of the culture medium was: $CaCl_2$: 1.8×10^{-4} M; $MgSO_4$: 1.7×10^{-3} M; $(NH_4)_2SO_4$: $7.6 \times 10^{+3}$ M; dissolved in bidistilled water and adjusted to pH 7.0 with NaOH 0.1 N. [27].

MEDIUM	E_{pit} (V, SCE)	pH	MYCELIUM (mg)
NaCl 10^{-4} M	-0.20	7.00	—
NaCl 10^{-3} M	-0.40	7.00	—
NaCl 10^{-2} M	-0.56	7.00	—
sterile	-0.37	7.00	—
C. resinae	-0.44	5.20	8
C. resinae	-0.48	4.20	20
C. resinae	-0.51	3.20	not measured
C. resinae	-0.53	2.90	150

Mansfeld et al. [4] showed that E_{pit} for 304SS in deaerated 3.5% NaCl decreased from about +300 mV vs. SCE at pH 8 to about 0 mV at pH 2. Since E_{pit} becomes more negative with increasing acidity and E_{corr} must be more negative than E_{pit} as long as pitting does not occur, localized acidification by microorganisms within a biofilm cannot be used to explain shifts of E_{corr} in the positive direction for stainless steels in natural seawater.

Salvarezza and Videla [27] demonstrated the relationship between E_{pit} values, chloride concentration and fungal growth on 2024 aluminum surfaces–Table 1. As the chloride concentration of sterile media increased, E_{pit} moved to more negative values in agreement with Equation 2. Fungal growth resulted in a marked decrease of medium pH and a corresponding decrease of E_{pit}.

Polarization resistance (R_p), defined as the slope of a potential (E) vs. current density curve at E_{corr} is inversely proportional to the corrosion current density (i_{corr}):

$$R_i = b_a b_c / 2.3 (b_a + b_c) i_{corr} \tag{4}$$

where b_a and b_c are the anodic and cathodic Tafel slopes, respectively. Since R_p is determined in a narrow range around E_{corr}, the polarization resistance technique is essentially a non-destructive technique which can be used repeatedly on the same sample without altering the properties of the biofilm and/or the metal surface. Nivens et al. [28] estimated i_{corr} from experimental R_p data for 304SS exposed to a seawater medium containing Vibrio natriegens and concluded that the corrosion rate increased with the bacterial production of extracellular polymer. Little et al. [19] determined the dependence of E_{corr} and R_p on the rotation speed of a rotating cylinder electrode for five Cu based materials and concluded that the anodic reaction was under mixed charge transfer and mass transport control. Little and Mansfeld [20] have given a more detailed

analysis of polarization resistance measurements for Cu-based materials exposed to natural and artificial seawater. R_p, b_a and b_c were calculated for each exposure time from polarization curves recorded within ± 30 mV of E_{corr}. This approach allows a quantitative calculation of i_{corr} according to Equation 4 and an evaluation of the changes of i_{corr}, b_a and b_c and E_{corr} with exposure time.

A simplification of the polarization resistance technique, linear polarization [29], assumes a linear relationship between potential (E) and current density (i) in a narrow range around E_{corr}. This approach is used in field tests and forms the basis of commercial corrosion rate monitors.

Dual Cell Techniques

The dual-cell, split-cell or biological battery [30–32] allows continuous monitoring of changes in corrosion rates due to the presence of a biofilm. In this technique two identical electrochemical cells are biologically separated by a semipermeable membrane. The two working electrodes are connected to a zero resistance ammeter or a potentiostat set to an applied potential of 0 mV. Bacteria are added to one of the two cells and the sign and magnitude of the resulting galvanic current is monitored to determine details of the corrosive action of the bacteria. Daumas et al. [32] used this technique to study corrosion of K55 steel in the presence of hydrogenase-containing (Hase$^+$) and non-hydrogenase (Hase$^-$) SRB. During growth of the Hase$^+$ microorganisms, electrons resulting from the metal oxidation reaction at the sterile electrode flowed to the inoculated electrode which had become the cathode as a result of the acceleration of the cathodic reaction on that electrode. The authors concluded that the oxidation of the cathodic hydrogen by the bacteria was the dominant mechanism for the enhanced corrosion rate. In the presence of Hase$^-$ SRB the colonized electrode became the anode due to stimulation of the anodic reaction by H_2S. Little et al. [12] used the dual cell technique to demonstrate that the sign and the magnitude of corrosion currents resulting from marine biofilms on 304SS varied with the exposure conditions (light vs. dark) and the biological composition of the biofilm–Figure 8.

Electrochemical Impedance Spectroscopy

Electrochemical impedance spectroscopy (EIS) or ac impedance is a technique for determining properties of metallic surfaces by applying a small ac signal [4] over a wide frequency range. Dowling et al. [33] used EIS to study the effects of mixed cultures of bacteria on the corrosion of carbon steels in marine environments. A comparison of the corrosive effect of *Vibrio natriegens* in batch culture with that of a sterile chamber over a three day period showed that bacteria increased the corrosion rate–Figure 9. Examination of the impedance behavior of a continuous flow system with low carbon levels showed that the corrosion rate was comparable between samples with and without bacteria.

Formation of biofilms and calcareous deposits on stainless steels and titanium during exposure to natural Pacific Ocean water was followed by Mansfeld et al. using EIS [11]. The impedance spectra for three stainless steels and titanium did not change as a function of alloy composition, exposure time or biofilm formation and remained capacitive
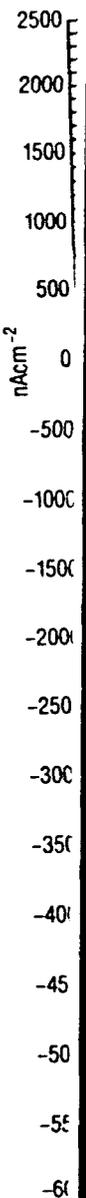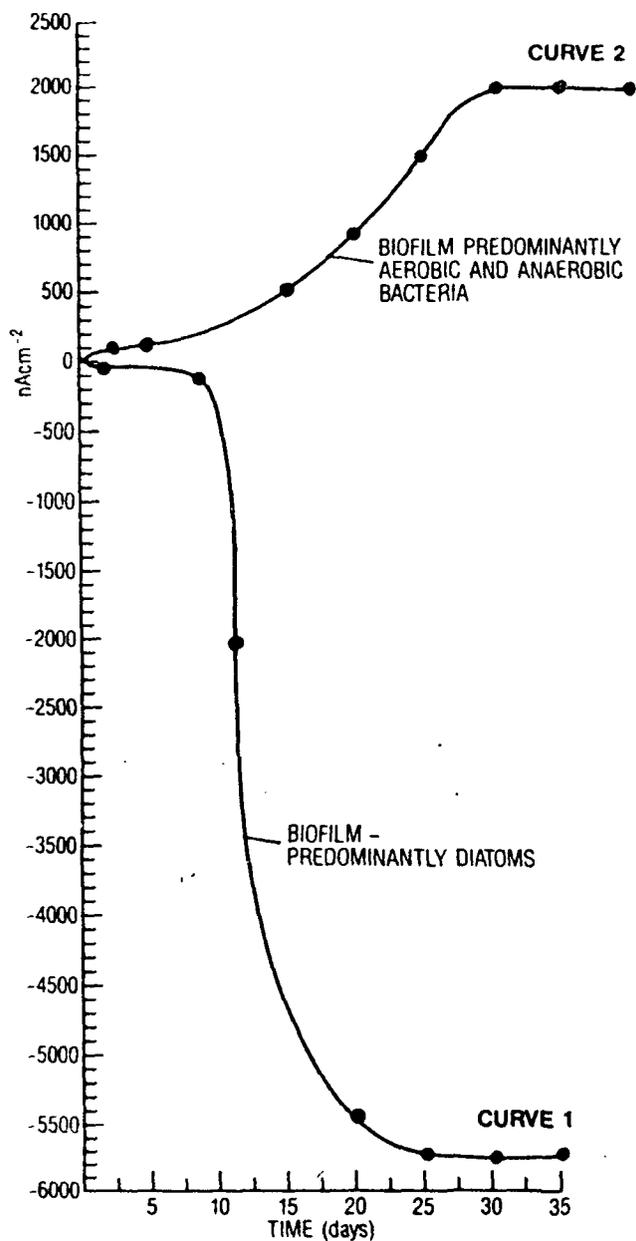
Figure 8. Galvanic current measured with dual cell for 304SS exposed to Gulf of Mexico water [12].
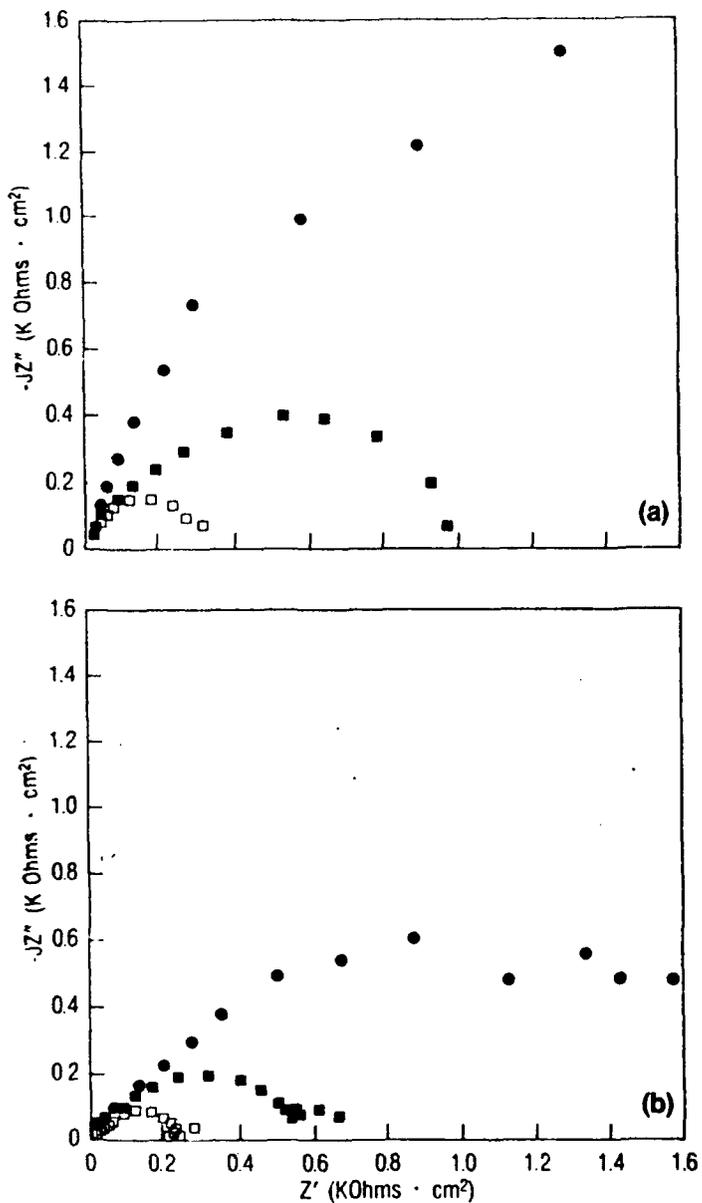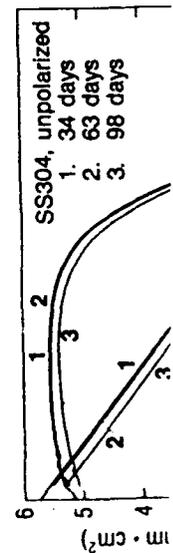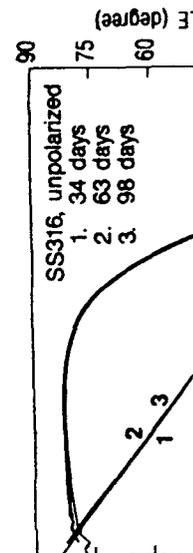
Figure 9. EIS data obtained at (a) initial time (■) and after three days with (□) and without (●) V. natriegens in batch culture, and (b) after three days with the vibrio in continuous flow followed by 24 hours in batch culture [33].
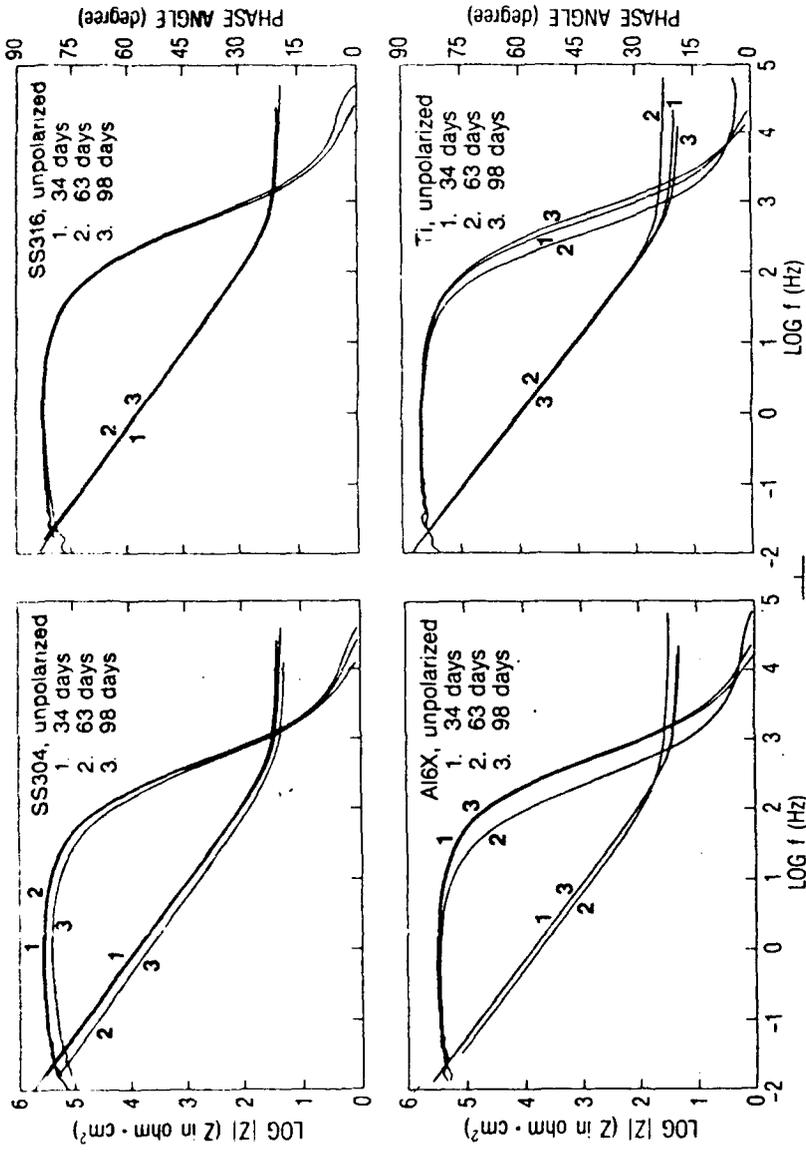
Figure 10. Bode plots for 3 stainless steels and titanium exposed to natural Pacific Ocean water [11].
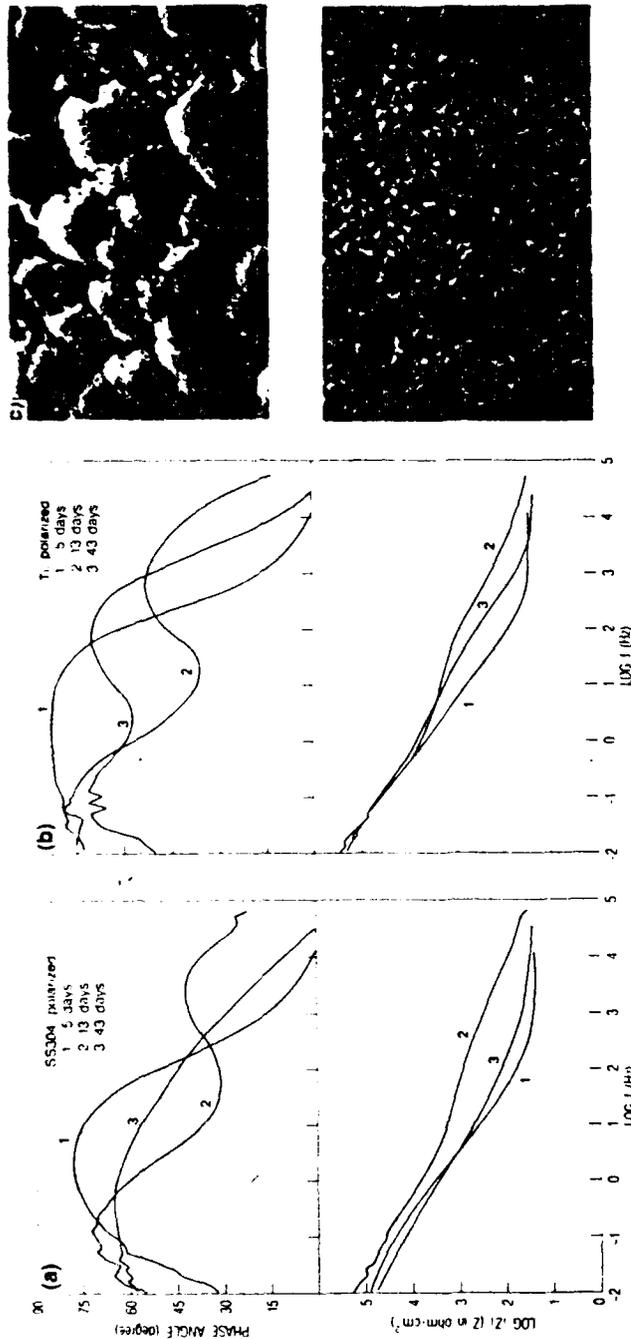
Figure 11. Bode plots for alloys polarized to -850 mV (SCE) as a function of exposure time to flowing natural seawater (a) 304SS, (b) titanium grade 2, and (c) surfaces after polarization at -850 mV (SCE) in flowing natural seawater for 43 days [11].

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over a four month period–Figure 10. EIS did provide information about the growth and structure of calcareous deposits on cathodically polarized surfaces–Figure 11a, 11b and 11c. Little et al. [19,20] used EIS to follow biofilm formation on five copper-based materials at the same Pacific Ocean site. Impedance spectra varied with alloy composition, changed as a function of exposure time and were altered by the presence of a biofilm. The complexity of impedance spectra (Figure 12a and 12b) was attributed to the formation of corrosion products and biofilms, and the contributions of charge transfer and mass transport reactions mediated by the layers.

Electrochemical Noise Analysis

Statistical analysis of noise fluctuations in potential or current data can provide information as to the nature of corrosion processes. Frequency and amplitude of fluctuations can be used to estimate corrosion rates. Moosavi et al. [34] presented noise plots for reinforced concrete exposed to a marine medium containing SRB–Figure 13a and 13b. The time record after 218 days for covered rebars revealed events that could be due to sudden rupture of the protective oxide film followed by immediate repassivation. Iverson et al. [35] obtained potential noise data in the laboratory for mild steel in a seawater culture of *Desulfovibrio* and in the field for a gas transmission line. The authors concluded that breakdown of iron sulfide films was accompanied by generation of electrochemical potential noise.

Surface Analysis

Methods for detecting and monitoring MIC have been reviewed by Stoecker [36], Tatnall [37], Pope [38], Tatnall et al. [39] and Galbraith and Lofgren [40]. Most detection and verification techniques involve inspection of corroded material, test coupons, or corrosion deposits for the detection of specific types and numbers of bacteria, (e.g., SRB, metal-depositing bacteria or acid-producing bacteria) or corrosive by-products, such as chlorides, acids or sulfides. Galbraith and Lofgren [40] described methods for culturing specific microorganisms and direct microscopic counts using fluorescent techniques. Tatnall et al. [41] developed a test kit for the detection and enumeration of SRB and prepared a comparative review of similar methods. Others [42] recommend a quantification of hydrogenase enzyme as a diagnostic tool to estimate the corrosivity of biofilms. Lewandowski et al. [21] developed microelectrodes for pH, dissolved oxygen and dissolved sulfide to probe the metal/biofilm interface and interfacial properties. Pope and Zintel [43] discussed chemical, microbiological and metallurgical methods for studying MIC in field and laboratory conditions.

Much of the information that has been collected for marine biofilms on metal surfaces has been derived from micrographs prepared by scanning electron microscopy (SEM) [44,45]. Preparation of biological material for the SEM requires fixation and dehydration through a series of solvents and coating with a metallic layer. Little et al. [46] demonstrated that SEM sample preparation introduces artifacts in both the areal coverage and composition of biofilms. Using an environmental scanning electron microscope (ESEM) that can accommodate wet, unfixed, uncoated biological material, they compared marine biofilms before and after fixation on stainless steel and copper surfaces–Figures 14 and 15, respectively. The wet marine biofilm on the stainless steel surface had a gelatinous
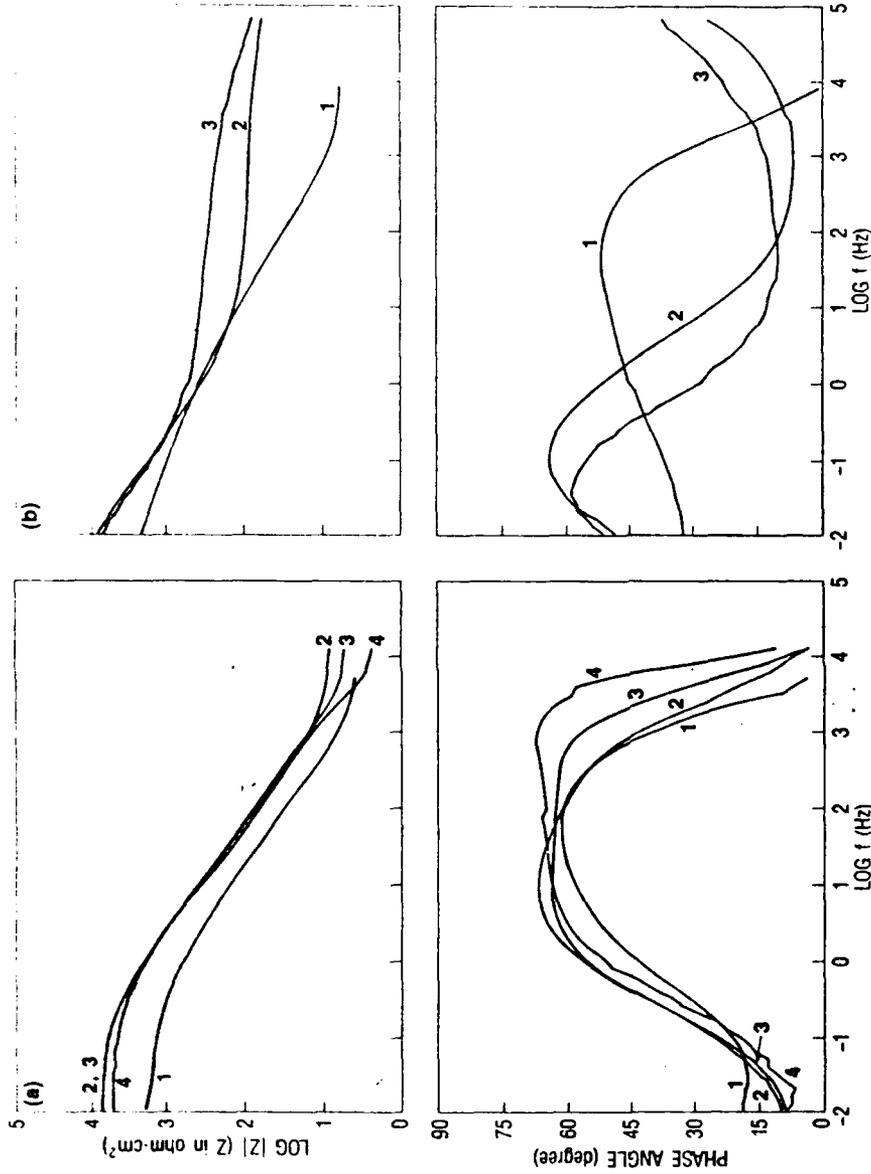
Figure 12. (a) Bode plots for 99 Cu as a function of exposure time to artificial seawater. Exposure times: 1 day (curve 1), 3 weeks (curve 2), 5 weeks (curve 3), and 8 weeks (curve 4); (b) Bode plots for 99Cu as a function of exposure time to natural seawater. Exposure times: 1 week (curve 1), 7 weeks (curve 2), and 10 weeks (curve 3).
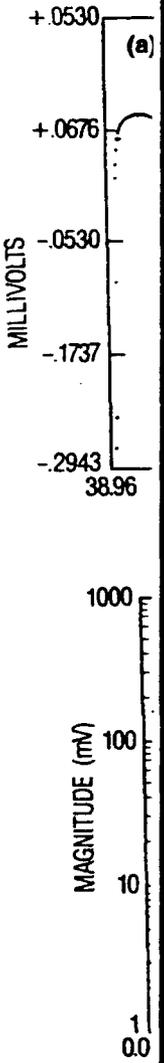
Figure 13.
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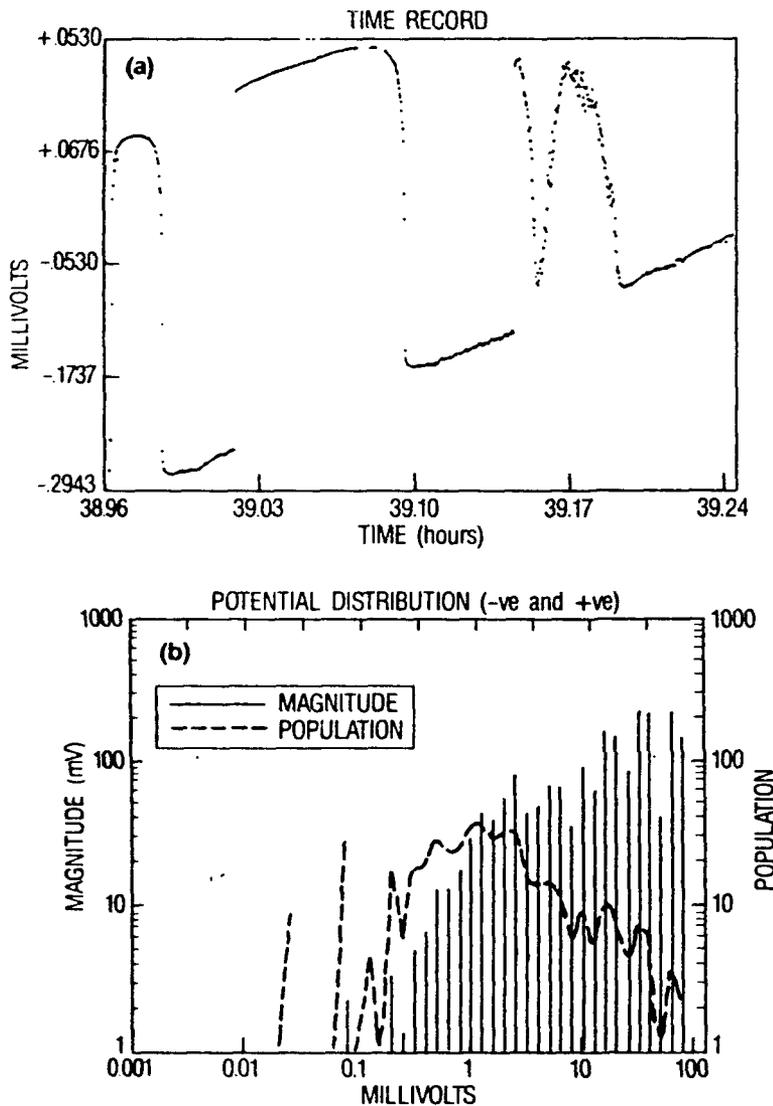monolayer of bacteri
alteration in the bic

Figure 13. Noise analyses (a) time record after 218 days for covered
reinforcements in media containing SRB and (b) potential distribution
chart showing population and magnitude of potential fluctuation [34].

appearance. Numerous microalgae could be distinguished throughout the gel, but it was impossible to image individual bacterial cells with the ESEM. After solvent removal of water, much of the extracellular polymeric material from the biofilm was also removed, decreasing the actual areal coverage of the biofilm on the surface and exposing a monolayer of bacterial cells, typically referred to as the biofilm. The most conspicuous alteration in the biological composition of the marine biofilms as a result of SEM
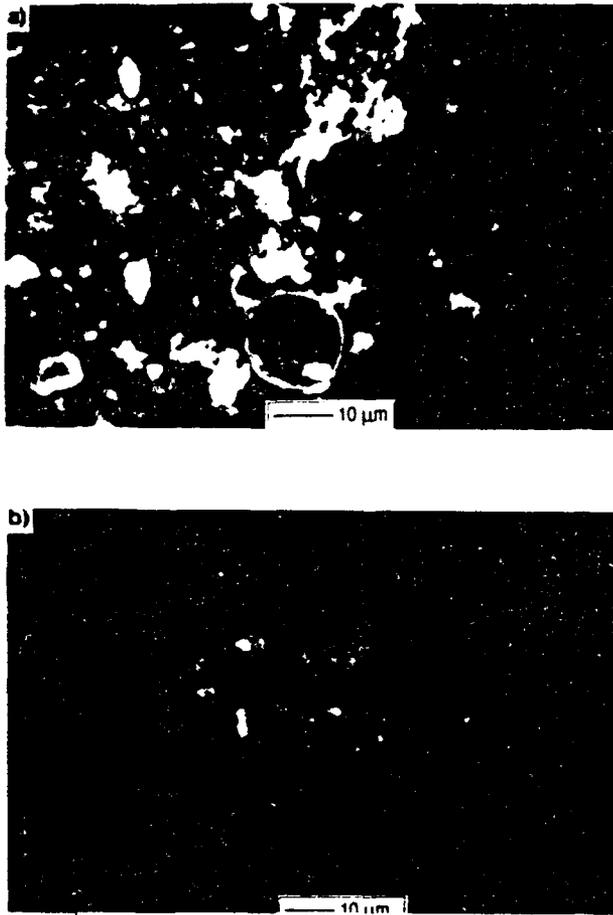
Figure 14. ESEM images (a) wet marine biofilms on stainless steel surface and (b) marine biofilms after treatment with acetone and xylene [46].

sample preparation was the partial removal of diatoms from the stainless steel surface and the complete removal of diatoms from the copper surface. It appears that the number of diatoms in marine biofilms and their potential for oxygen production within the biofilm are grossly underestimated by traditional SEM techniques.

The solvent removal of water removes the polymeric matrix and metals bound by the matrix–Tables 2 and 3. Extracellular polymeric material is predominantly negatively charged so that it can act as an effective cation exchanger, increasing local concentrations of heavy metals including Al, Ni and Ti. Dehydration of the biofilm with acetone
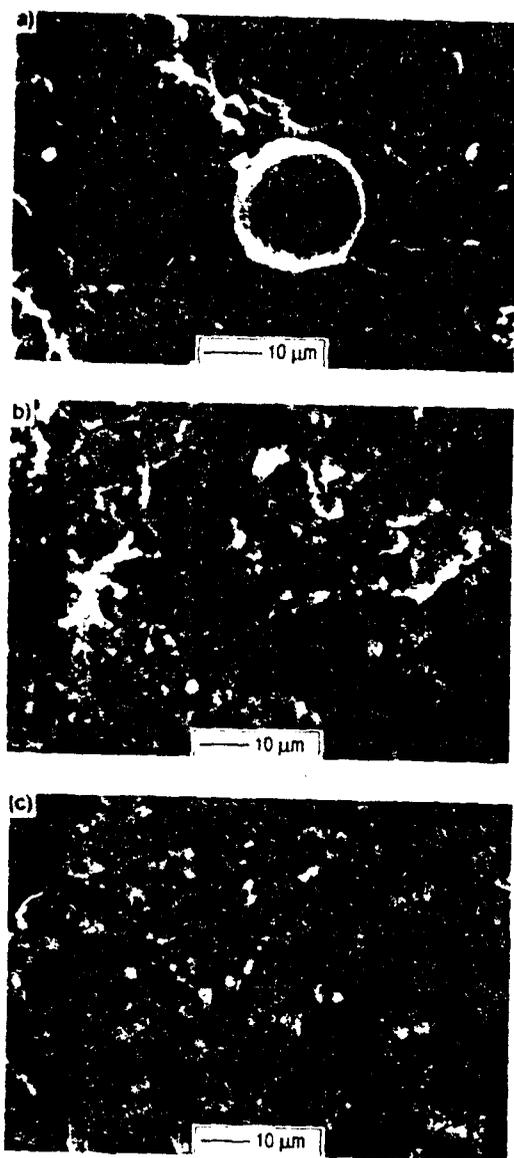

Figure 15.1
surface, (b
biofilm afte

Figure 15. ESEM images (a) wet marine biofilm containing diatom on copper surface, (b) marine biofilm after treatment with acetone, and (c) marine biofilm after treatment with acetone and xylene.

Table 2. Weight percent elemental composition of a biofilm on 304SS surface [46].

ELEMENT	BASE METAL	AFTER EXPOSURE TO SEAWATER	AFTER XYLENE
Fe	72.55	11.24	72.43
Cr	18.10	4.71	18.17
Ni	8.02	34.00	7.78
Si	.93	7.35	1.06
Al	.40	3.33	0.57
Cl		0.66	
S		0.99	
K		1.88	
Na		2.12	
Mg		4.98	
Ti		28.74	

Table 3. Weight percent elemental composition of a biofilm on a copper alloy surface [46]..

ELEMENT	Cu IN H_2O	Cu AFTER ACETONE	Cu AFTER XYLENE
Al	9.49	1.22	0.74
Si	21.38	1.89	1.07
Cl	0.93	15.9	15.93
Cu	59.62	80.99	82.06
Mg	1.96		
P	0.98		
S	0.95		
Ca	0.49		
K	0.67		
Fe	3.52		

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and xylene either extracts bound metals from the biofilm, alters the electrostatic charges within the biofilm or removes the metals with the extracellular polymeric material. The solvent effects of acetone and xylene cannot be separated from the mechanical disturbance to the biofilm during sample manipulation.

CONCLUSIONS

Polarization techniques are useful for a survey of the corrosion kinetics in a large potential region. For studies of MIC, pitting potential has been used to assess effects of microbes on localized corrosion. Drastic changes in the polarization behavior of copper alloys have been observed in the presence of sulfides and SRB. Polarization resistance and linear polarization techniques have been useful for determining

instantaneous corrosion rates and monitoring corrosion behavior as a function of exposure time. EIS has emerged as a technique for characterization of surface properties of metals exposed to marine environments. Since EIS is a non-destructive technique, it can be used to determine corrosion mechanisms as well as to follow changes in corrosion rates. Similarly, electrochemical noise is non-destructive since it does not require the imposition of an external signal. However, its use has been limited so far to qualitative MIC studies. As in all studies of corrosion phenomena, combinations of electrochemical, surface analytical and microbiological techniques are needed to provide quantitative mechanisms of MIC.

ACKNOWLEDGMENTS

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